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MxA Protein in Capillary Blood of Children With Viral Infections

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Capillary blood of febrile children was lysed by using a lysis buffer containing ascorbic acid. MxA quantitation was performed by an immunochemiluminescent assay. The MxA values were significantly higher in capillary blood of infants with viral infections due to adenovirus (n = 5), rotavirus (n = 15), or respiratory syncytial virus (n = 28), than in capillary whole blood from infants with bacterial infections (n = 6) and healthy control patients (n = 20). A strong correlation was found between the MxA values in capillary whole blood and peripheral whole blood ($r' = 0.86$, $P < 0.0001$, n = 48). The MxA values found at these two sites were compared with the levels of IFN-α obtained by a dissociation enhanced lanthanide fluoroimmunoassay. A correlation between these two values was found. The results show that the combination of collection of blood by finger prick and specific immunochemiluminescent assay for MxA protein measurement may be of value for the diagnosis of viral infections in children. ***J. Med. Virol. 59:547–551, 1999.***

KEY WORDS: acute viral diseases; MxA protein; febrile infants; capillary blood; immunochemiluminescence; IFN-α

INTRODUCTION

Interferon-α (IFN-α) is predominantly produced in response to viral infections and contributes to host defense by establishing an antiviral state in target cells [Taylor and Grossberg, 1990; Belardelli, 1995]. Among the IFN-α–induced proteins, MxA is remarkable for its high level of expression in PBMC cytoplasm [Horisberger and Hochkeppel, 1987]. Moreover, the MxA protein is specifically induced in a dose-dependent manner by type I IFNs in vitro and in vivo, whereas IFN-γ enhanced the level of other induced proteins such as p68 kinase (p68K) or 2′–5′ oligoadenylate synthetase (2′–5′ OAS) [Buffet-Janvresse et al., 1983; von Wussow et al., 1990; Simon et al., 1991]. By measuring IFN-induced proteins, the presence of biologically active IFNs is detected more consistently than that of circulating IFN-α in serum of patients with viral infections [Roers et al., 1994]. As a matter of fact, the half-lives of different IFN-αs are short and the most commonly used assays for the biological activity of interferon involve fastidious manipulations [Joklik, 1990; Taylor and Grossberg, 1990; Belardelli, 1995]. Therefore, in order to detect IFN-α more easily, immunoassays that improve the availability of IFN-α quantitation have been developed, even though the level of circulating IFN-α does not reflect the biological activity of IFN-α in target cells [Cederblad et al., 1998].

Since MxA protein is specifically induced by type I IFNs, some authors have used this approach to study the IFN-α state in patients with viral infections or in IFN-α–treated patients [Jakschies et al., 1990; Bezares et al., 1996; Halminen et al., 1996]. Nevertheless, the assays used in these studies are based on immunoblotting or immunofluorescence, and so they are time-consuming and can be performed in specialized clinical laboratories only. Towbin et al. [1992] and Oh et al. [1994] improved the MxA protein quantitation assay in terms of time and number of manipulations. The assays developed by these authors are based on immunoenzymology and immunochemiluminescence, and measure MxA in peripheral whole blood lysates. Recently, we reported the clinical value of measuring MxA in peripheral venous blood by an immunochemiluminescent assay [Chieux et al., 1998]. Since technical problems were reduced for MxA quantitation, MxA protein

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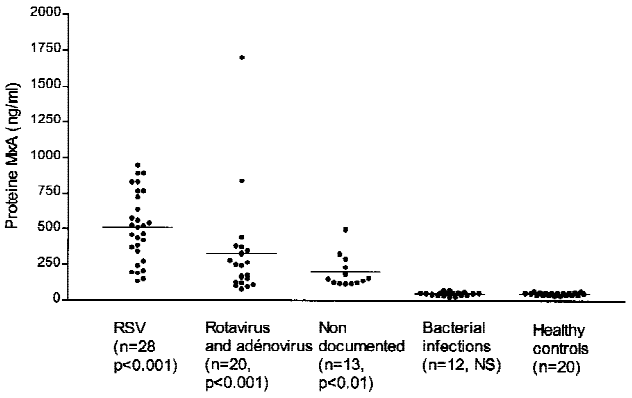

Fig. 1. Levels of MxA protein in capillary blood of children. Twelve patients had bacterial infection including 11 cases pyelonephritis caused by *Escherichia coli* confirmed by blood and urine culture and 1 case of meningococcal meningitis confirmed by CSF culture. Patients with suspected viral infection are patients with symptoms of infection but no biological evidence of bacterial infection. The MxA quantitation was performed by using an immunochemiluminescent assay. The mean of each group is represented. The *P* values versus healthy controls are shown with the group size. NS: not significant.

could be a useful marker for rapid diagnosis of viral infections. However, difficulties in obtaining peripheral venous blood are often encountered in infants. This practice is also resource-consuming in terms of personnel and by the need to transport the samples. In the present report, we compare the levels of MxA and IFN-α in peripheral blood of patients with viral diseases, and we show that capillary whole blood can be used to measure MxA levels in infants with suspected viral infections.

MATERIALS AND METHODS

Patients

Blood samples were collected on admission to Féron-Vrau Hospital, Lille, France. The patients were suffering from acute viral and bacterial infections (n = 12) and presented clinical symptoms as described in Figure 1 (M/F ratio was 34/20; age 1–24 months with a mean of 10.5 months). The diagnosis of viral infection was based on the association of clinical symptoms and the detection of viruses. Rotavirus and adenovirus were detected in stools by using a latex agglutination (Meritec-Rotavirus, Meridian Diagnostics, Cincinnati, OH, and Adenolex, Orion Diagnostica, Espoo, Finland, respectively), RSV was detected in nasopharyngeal fluids by using an indirect immunofluorescent method (Argene, Biosoft, Vérilhes, France).

A sample of blood was obtained from each of the 13 patients with suspected acute viral infection on their admission to hospital. The clinical symptoms were gastroenteritis for seven children and bronchiolitis for six infants. No virus was found in stools or in nasopharyngeal fluids but C reactive protein (CRP), currently used as a bacterial infection marker, was below 20 mg/l for each of these patients.

A sample of whole blood was obtained from 20 children without any apparent symptoms of infection 2 weeks before and after blood sampling (M/F ratio was 12/8; age 1–5 months with a mean of 3 months). The children's parents gave their consent for drawing blood.

Sample Processing

Peripheral whole blood was drawn by venipuncture into 2-ml tubes containing lithium heparin and into 2-ml tubes without anticoagulant (Becton Dickinson, Le Pont de Claix, France). Fifteen microliters of heparinized whole blood samples were added to 285 μl of ascorbic acid lysis buffer (ACS: 180, Chiron Diagnostics, Les Ullys, France) following a previously described simplified lysis procedure [Chieux et al., 1998]. The tubes without anticoagulant were rapidly centrifuged and the sera were decanted into sterile plastic tubes. When necessary, the lysates and sera were stored at −80°C until assay.

Simultaneously, 60 μl of capillary blood collected in heparinized microtubes were obtained by finger prick (Microcap, Chiron Diagnostics, Villeneuve D'Ascq, France). The capillary tubes were capped as soon as they were filled. The microtubes were then placed in ice-cold water and taken to the laboratory. To resuspend the blood cells, the capillary blood was mixed by gently rolling the tubes between two fingers. The whole blood was then ejected out of the tube in lysis buffer by using a laboratory bulb. The capillary blood was diluted 20-fold in ascorbic lysis buffer and lysed by using the procedure described above. When necessary, the lysates were stored at −80°C until assay.

Quantitation of MxA Protein

Two hundred μl of test samples, standards and controls, were incubated in plastic tubes simultaneously with 100 μl of Lite reagent (N-hydroxysuccinimide–activated dimethyl acridinium ester-labeled detector antibody) and 200 μl of solid phase (Paramagnetic particles-conjugated antibody; Chiron Diagnostics, Alameda, CA). The tubes were then shaken by using a Multi-tube Vortexer (Model 4010, Chiron Diagnostics) for 3 min at room temperature. These mixtures were then incubated 30 min at 37°C. After incubation, the solid-phase-bound immune complex was separated with a magnetized separator rack, Magic Rack (Chiron Diagnostics) for 3 min at room temperature. The separated pellets were then resuspended in 1 ml of distilled water using the Multi-tube Vortexer. The unbound antigen or antibody was discarded by decanting the rack. The separated pellets were then washed once more with 1 ml of distilled water as described above. The pellets were resuspended in 100 μl of distilled water. The relative luminescence units (RLUs) were counted by using an MLA-I luminometer (Chiron Diagnostics). The RLUs were converted in ng/ml by using a master curve.

Quantitation of IFN-α

Circulating IFN-α was simultaneously quantitated in the sera of 28 consecutive patients and controls by a specific and sensitive dissociation-enhanced lan-

thanide fluoroimmunoassay (DELFIA) based on the direct sandwich technique using a mixture of two murine monoclonal antibodies (MoAbs) to human IFN-α LT27:273 and LT27:293, which bind more than 90% of natural IFN-α subtypes coated onto microtiter plate wells (LKB Wallac, Turku, Finland), and an europium-labeled murine antihuman IFN-α as previously described [Cederblad et al., 1998]. Samples (100 μl) diluted with an equal volume of dilution buffer containing irrelevant mouse monoclonal IgG1:B1:2 at 50 μl/ml were then added to the plate. After 2 hr, the plates were washed three times and 0.2 ml of the europium-conjugated antibody at 1/800 dilution was added per well in dilution buffer. After 1 hr, the plates were washed six times and 0.2 ml of enhancement solution (LKB Wallac, Turku, Finland) was added per well to promote the dissociation of Eur^{3+} cations from the labeled antibody into solution, where they formed fluorescent chelates with components of the enhancement solution. After 20 min, the fluorescence in the microtitration strip wells was measured in a time-resolved fluorometer (1230 Arcus Fluorometer, LKB Wallac, Finland). The leukocyte reference interferon G-23-902-530 (National Institute of Health) was used as standard.

Statistical Analysis

Comparisons of quantitative parameters were made by using the nonparametric Mann-Whitney U test. The correlation between the MxA levels in capillary and peripheral whole blood lysates and the correlation between MxA protein and IFN-α levels were studied by using the Spearman's test. The results are represented as mean standard deviation (SD).

RESULTS

MxA is an intracellular protein; therefore, a lysis procedure was used for the treatment of both capillary and peripheral blood. MxA levels in capillary blood from healthy infants did not differ from those found in peripheral blood from healthy adults (57 ± 21 ng/ml, n = 20, vs. 45 ± 15 ng/ml, n = 30; $P > 0.1$). A large range of values was obtained in each group of patients with acute viral infection. In capillary whole blood lysates of patients with viral infections and of patients with suspected viral infections, the MxA levels were found to be higher than in whole blood lysates from the patients with bacterial infection and from the control patients (Fig. 1). In order to investigate further the value of measuring MxA levels in capillary blood, it seemed judicious to us to compare the levels of MxA in capillary and peripheral venous blood. MxA levels were significantly higher in peripheral whole blood lysates than in capillary blood (576 ± 474 ng/ml vs. 436 ± 309 ng/ml, $P = 0.001$, n = 48). However, the MxA levels in capillary blood lysates of virally infected patients were much higher than those in capillary blood and peripheral blood lysates of healthy controls (436 ± 309 ng/ml vs. 57 ± 21 ng/ml, $P < 0.001$, and 436 ± 309 ng/ml vs. 60 ± 14 ng/ml, $P < 0.001$, respectively). A high correlation ($P < 0.001$) was observed between MxA levels in peripheral and capillary blood samples (Fig. 2). Due to the wide range of MxA values (25 to 2,092 ng/ml), these are expressed in logarithmic format in Figure 2.

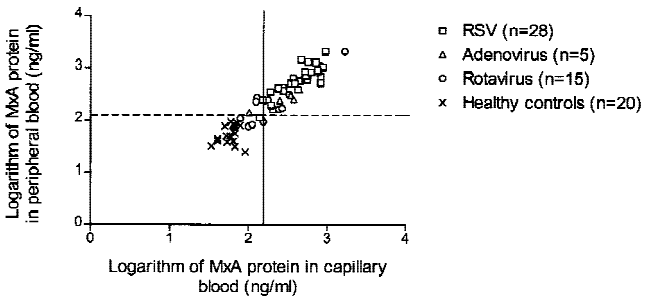

Fig. 2. Correlation between the MxA concentrations in capillary blood and in peripheral blood of 48 patients with various documented viral infections ($r' = 0.86$, $P < 0.0001$, n = 48). The dotted line and the continuous line represent the mean + 2 × SD values of 20 control samples obtained in capillary and peripheral blood respectively.

The MxA protein is specifically induced by type I IFNs. Therefore, when possible, the MxA levels were compared with the concentrations of IFN-α. The levels of IFN-α in sera of patients with acute viral infection were significantly higher than those of the controls (17.6 ± 21.9 IU/ml, n = 23, vs. 2.2 ± 0.4 IU/ml, n = 10, respectively, $P < 0.05$). Moreover, a correlation was found between the IFN-α levels and the MxA concentrations in peripheral blood and in capillary blood ($P < 0.01$ and $P < 0.01$, respectively). Nevertheless, the detection of MxA proteins in capillary blood and peripheral blood appears to be more sensitive than IFN-α quantitation. Indeed, the levels of MxA in each patient were higher than the mean + 2 × SD of those of controls, whereas in 5 of 23 patients, the levels of IFN-α were lower than the mean + 2 × SD of those of controls. The MxA and IFN-α values are expressed in logarithmic format in Figure 3.

DISCUSSION

Because type I IFNs and certain viruses can directly activate the synthesis of MxA mRNA, a rapid detection of MxA may be a useful test for diagnosing viral diseases [Fäh et al., 1996; Halminen et al., 1997]. The aim of some authors was to simplify the assays for detection of MxA protein in whole blood [Towbin et al., 1992; Oh et al., 1994]. In one of our previous studies, it was demonstrated that MxA protein detection by using immunochemiluminescence might be a sensitive and convenient method for the diagnosis of viral diseases [Chieux et al., 1998]. Capillary blood has been used extensively for screening studies in infants, especially in neonatalogy, but this is the first report of MxA levels in capillary blood of febrile children. As the blood collection by finger prick is accepted more readily than the peripheral venous puncture, it can be applied to children more easily. The use of lysis buffer containing ascorbic acid is particularly adapted to the treatment of blood drawn in microtube, and all the blood cells were ex-

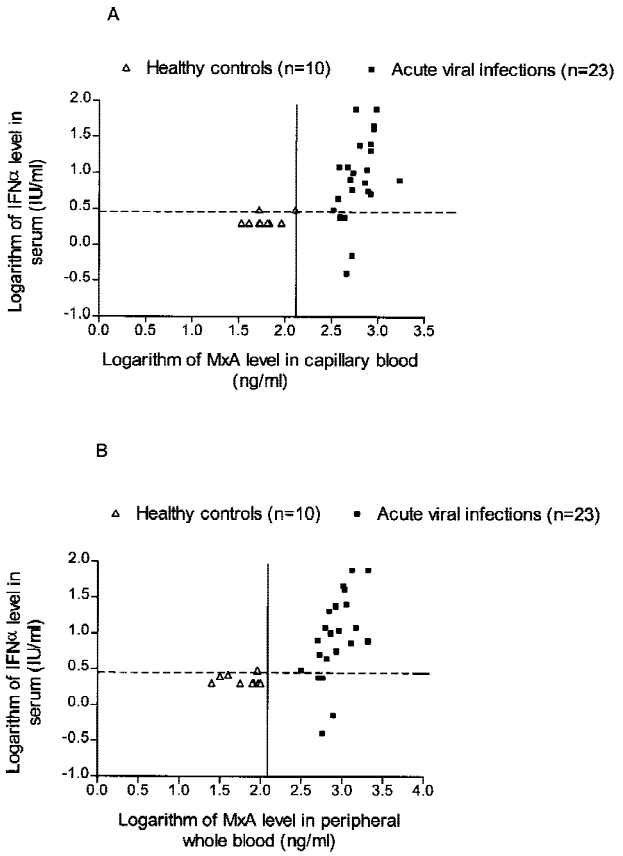

Fig. 3. Correlation between the MxA levels in capillary blood (**A**) and peripheral blood (**B**) lysates and the IFN-α concentrations detected in sera of 23 patients with documented viral infection ($r' = 0.77$, $P < 0.001$, and $r' = 0.98$, $P < 0.001$, respectively). IFN-α was quantitated by using a dissociated-enhanced lanthanide fluoroimmunoassay. In both figures, the continuous line represents the mean + 2 × SD of MxA protein, and the dotted line represents the mean + 2 × SD of IFN-α level of 10 healthy controls.

tracted out of the capillary tube by the lysis buffer. In spite of the fact that the MxA protein levels detected in capillary blood are lower than those obtained in peripheral blood lysates, the two methods developed here were shown to be reliable.

The biological half-life of IFN-α is very short (1–2 hr). In contrast, the half-life of MxA is rather long (2.5 days) and it may indicate the presence of biologically active IFN that escaped direct measurement.

Higher MxA levels in capillary blood were found in patients with localized viral infections due to RNA viruses (RSV and rotavirus) and DNA viruses (adenovirus), but not in the blood of patients with bacterial infections. Interestingly, other experiments revealed that the MxA amounts in peripheral whole blood were correlated with CMV-related diagnostic parameters in three allograft patients with clinical symptoms of CMV infection [Chieux et al., 1998]. Further investigation is needed to study the MxA values in capillary whole blood of patients with disseminated viral infections.

Dissociated-enhanced lanthanide fluoroimmunoassay (DELFIA) reproducibly determines IFN-α levels in serum [Ronnblom et al., 1997]. The DELFIA is more convenient than the current bioassay, but it does not explore the biological activity of IFN-α. In this study, it was evidenced that the detection of MxA protein might be a reliable and complementary marker of biological activity of IFN-α. However, as shown by the current study, the detection of MxA protein is more frequent than the detection of IFN-α by using the DELFIA test, which is probably due to the fact that the biological half-life of MxA protein is much higher than that of IFN-α.

Our results and those provided by other authors are in favor of the value of MxA as a marker of viral disease [Fäh et al., 1995; Halminen et al., 1997; Chieux et al., 1998]. Halminen et al. [1997] studied the MxA levels in PBMCs isolated from blood of infants with viral infections by using FACs cytometer. This method is sensitive but laborious and time-consuming; it requires PBMCs isolation and also involves specialized laboratory equipment for measurement. Moreover, the amount of heparinized venous blood required for MxA detection ranged between 3 and 5 ml, whereas only 60 microliters were needed for MxA quantitation by using immunochemiluminescence. The method used in the present report may be useful in clinical laboratories since the combination of capillary blood collection and detection of MxA protein by immunochemiluminescent assay is rapid and convenient and does not require trained staff or specialized laboratory equipment.

Since the measurement of MxA is easy and discriminates between bacterial and viral infections, increased MxA protein expression levels can be used in differential diagnosis of bacterial versus viral infection [Cohen et al., 1992].

Type I interferons are synthesized and secreted in response to viral infections. In several chronic inflammatory diseases of unknown etiology, such as multiple sclerosis or diabetes mellitus, a viral cause or viral triggering has been discussed [Challoner et al., 1995; Andreoletti et al., 1997; Perron et al., 1997]. Since the MxA protein detected by using immunochemiluminescence is a simple and reliable marker of the presence of a virus, it may be useful for investigating the role of viruses in these conditions.

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REFERENCES

Andreoletti L, Hober D, Hober-Vanderberghe C, Belaich S, Vantighem MC, Lefebvre J, Wattré P. 1997. Detection of coxsakie B virus RNA sequences in whole blood samples from adult patients at the onset of type I diabetes mellitus. J Med Virol 52:121–127.

Belardelli F. 1995. Role of Interferon and other cytokines in the regulation of the immune response. APMIS 103:161–179.

Bezares F, Kohan S, Sacerdote de Lustig E, Farias E, Murro H, Horisberger MA. 1996. Treatment strategies for early-stage chronic lymphocytic leukaemia: can interferon-inducible MxA protein and tumour necrosis factor play a role as predictive markers for re-

sponse to interferon therapy. J Interferon Cytokine Res 16:501–505.

Buffet-Janvresse C, Magard H, Robert N, Hovanessian AG. 1983. Assay and the levels of 2′–5′ adenylate synthetase in lymphocytes of patients with viral, bacterial and autoimmune diseases. Ann Immunol 134:247–258.

Cederblad B, Blomberg S, Vallin H, Perers A, Alm GV, Ronnblom L. 1998. Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-alpha-producing cells. J Autoimmunity 11:465–470.

Challoner PB, Smith KT, Parker JD, MacLeod DL, Coulter SN, Rose TM, Schultz ER, Bennet L, Garber RL, Chang M, Schad PA, Stewart PM, Nowinski RC, Brown JP, Burmer GC. 1995. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. Proc Natl Acad Sci USA 92:7740–7744.

Chieux V, Hober D, Harvey J, Duhamel M, Lion G, Lucidarme D, Forzy G, Cousin J, Ducoulombier H, Wattré P. 1998. The MxA levels in whole blood lysates of patients with viral infections. J Virol Methods 70:183–193.

Cohen ML. 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257:1050–1055.

Fäh J, Pavlovic J, Burg G. 1995. Expression of MxA protein in inflammatory dermatoses. J Histochem Cytochem 43:47–52.

Halminen M, Ilonen J, Julkunen I, Ruuskanen O, Simell O, Mäekela MJ. 1997. Expression of MxA protein in blood lymphocytes discriminates between viral and bacterial infections in febrile children. Pediatric Res 41:647–650.

Horisberger MA, Hochkeppel KH. 1987. IFN-α–induced human 78 kD protein: purification and homologies with the mouse Mx protein, production of monoclonal antibodies, and potentiation effect of IFN-α. J Interferon Res 7:331–343.

Jakschies D, Hochkeppel HK, Horisberger MA, Deicher H, von Wussow P. 1990. Correlation of the antiproliferative effect and the Mx-homolgous protein induction by IFN in patients with malignant melanoma. J Invest Dermatol 95:238–241.

Joklik WK. 1990. Interferons. In Fields BN, editor. Virology, 2nd ed. Philadelphia: Lippincott-Raven, p 383–401.

Oh SK, Luhowskyj S, Witt P, et al. 1994. Quantitation of Interferon-induced Mx protein in whole blood lysates by an immunochemiluminescent assay: elimination of protease activity of cell lysates in toto. J Immunol Methods 176:79–91.

Perron H, Garson A, Bedin F, Beseme F, Paranhos-Baccala G, Komurian-Pradel F, Mallet F, Tuke PW, Voisset C, Blond JL, Lalande B, Seigneurin JM, Mandrand B. 1997. Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. Proc Natl Acad Sci USA 94:7583–7588.

Roers A, Hochkeppel HK, Horisberger MA, Hovanessian A, Haller O. 1994. MxA gene expression after live virus vaccination: a sensitive marker for endogenous type I interferon. J Inf Dis 169:807–813.

Ronnblom LE, Perers A, Vallin HS, Eriksson I, Osterlind A, Cederblad B, Alm G. 1997. Detection of serum interferon-alpha by dissociation-enhanced lanthanide fluoroimmunoassay: studies of patients with acute viral and bacterial infections. APMIS 7:531–536.

Simon A, Fäh J, Haller O, Staeheli P. 1991. Interferon-regulated Mx genes are not responsive to interleukin-1, Tumor Necrosis Factor, and other cytokines. J Virol 65:968–971.

Taylor JL, Grossberg SE. 1990. Recent progress in Interferon research: molecular mechanisms of regulation, action and virus circumvention. Virus Research 15:1–26.

Towbin H, Schmitz A, Jakschies D, von Wussow P, Horisberger MA. 1992. A whole blood immunoassay for the Interferon-inducible human Mx protein. J Interferon Res 12:67–74.

von Wussow P, Jakschies D, Hochkeppel KH, Fibisch C, Penner L, Deicher H. 1990. The human intracellular Mx-homologous protein is specifically induced by type I Interferons. Eur J Immunol 20: 2015–2019.